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DECLARATION

I, Kazunori OKAMURA, of HIRAKI & ASSOCIATES, do solemnly and sincerely declare as follows:

1. That I am well acquainted with the English and Japanese languages and am competent to translate from Japanese into English.
2. That I have executed, with the best of my ability, a true and correct translation into English of Japanese Patent Application No. 2002-307535 filed on October 22, 2002, a copy of which I attach herewith.

This 16th day of November, 2006

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OIPE NOV 3 0 2006

[Designation of Document] Specification

[Title of the Invention] SOLUTION MIXING APPARATUS AND SOLUTION MIXING METHOD

[Claims]

[Claim 1] A solution mixing apparatus comprising:

a base plate member on which a probe-fixed substrate is provided;

a planar member provided so as to form a space to be filled with a sample solution on the substrate;

a sample feed port for feeding the sample solution to the substrate; and

an actuating means for causing a relative motion between the planar member and the base plate member.

[Claim 2] The solution mixing apparatus according to claim 1, wherein the actuating means causes the planar member to linearly reciprocate.

[Claim 3] The solution mixing apparatus according to claim 2, wherein the reciprocation rate of the planar member is between 0.9 and 30 sec/cycle.

[Claim 4] The solution mixing apparatus according to claim 2, wherein the distance that the planar member linearly reciprocates is twice or longer than that of a probe-fixed area on the substrate.

[Claim 5] The solution mixing apparatus according to claim 1, wherein the actuating means spins the planar member.

[Claim 6] The solution mixing apparatus according to claim 5, wherein the spin rate of the planar member is between 0.9 and 30 sec/cycle.

[Claim 7] The solution mixing apparatus according to claim 5, wherein the planar member has a circular form, the probe-fixed area on the substrate has a polygonal form, and the diameter of the circular plate is twice or longer than the diagonal line of the polygonal substrate.

[Claim 8] The solution mixing apparatus according to claim 5, wherein the planar member has a circular form, the probe-fixed area on the substrate has a circular form, and the diameter of the planar member is twice or longer than the diameter of the probe-fixed area on the substrate.

[Claim 9] The solution mixing apparatus according to claim 5, wherein the actuating means comprises a shaft and an auxiliary member, at least one joint is fixed between the shaft and the auxiliary member, between the auxiliary member and the planar member, or between the auxiliary member and the other auxiliary member when a plurality of auxiliary members are used, and the shaft rotates to thereby spin the planar member.

[Claim 10] The solution mixing apparatus according to claim 1, wherein the distance between the base plate member and the planar member is between 50,000 nm and 100,000 nm.

[Claim 11] The solution mixing apparatus according to claim 1, wherein the surface of the planar member that is brought into contact with the sample solution is hydrophilic.

[Claim 12] The solution mixing apparatus according to claim 1, which comprises a temperature control mechanism for maintaining the temperature of the sample solution at a given level.

[Claim 13] A method for mixing a solution, wherein a sample solution is introduced into a space between the probe-fixed substrate and the planar member, the planar member is caused to move relative to the base plate member on which the substrate is provided, and the flow rate of the sample solution is maintained at substantially the same level at any spot on the substrate.

[Claim 14] A solution mixing apparatus comprising:

an installation member holding therein a probe-fixed substrate;

a sample feed port for injecting a sample solution into the installation member; and

an actuating means for spinning the installation member so as to mix the sample solution via centrifugal force.

[Claim 15] The solution mixing apparatus according to claim 14, wherein the actuating means generates a centrifugal force for pressing the surface of the substrate, which is opposite from the probe-fixed surface held in the installation member, against the inner wall of the installation member.

[Claim 16] The solution mixing apparatus according to claim 14, wherein the spin rate of the base plate member is between 200 rpm and 400 rpm.

[Detailed Description of the Invention]

[0001]

[Field of the Invention]

The present invention relates to a solution mixing apparatus and a method for mixing a solution for efficiently performing chemical reactions, particularly reactions of biomolecules that interact with probes fixed on a substrate.

[0002]

[Prior Art]

As a means for screening for new drugs, DNA microarray techniques have been attracting attention in recent years. Such techniques are used for extensive applications, such as DNA nucleotide sequence analysis and gene expression analysis, for the purpose of identification of gene mutation associated with diseases. An array substrate used for such applications comprises several hundreds to several thousands probes aligned and fixed on the surface thereof. As probes for DNA microarrays, in general, single-stranded or double-stranded synthetic DNA having the same sequence as that of a normal gene is used. Further, analytical techniques involving the use of such array substrate can target not only DNAs but also proteins. In any case, a plurality of types of probes are fixed on the array substrate, and hybridization of each probe with a sample is performed, which enables the analysis of the presence or absence of nucleic acids or proteins in the sample or the occurrence of changes in the abundance thereof.

[0003]

In general, hybridization is carried out by trickling a sample-containing hybridization solution on the probe-fixed array substrate, covering the substrate with a glass cover in order to prevent the hybridization solution from vaporizing, mounting the substrate in a humidified container or sealed cassette, and maintaining the temperature at a constant level over a considerably long period of time (12 hours or longer). As a technique for homogeneously performing hybridization or shortening the duration of hybridization, it is known that a hybridization solution is shaken or agitated simultaneously with shaking. In recent years, a spinning apparatus for performing hybridization on a membrane or a solution shaking apparatus for performing hybridization on an array substrate is used.

[0004]

U.S. Pat. Publication No. 2001/0046702 describes an array hybridization apparatus having a substantially planar bottom 34, a cover 35, a fluid port 36, a space adjusting means 37 for adjusting the spacing between an array and the bottom surface, and an array installation member 38, as shown in Fig. 14. In the subject apparatus, an array is placed on the space adjusting means in the chamber 39, and the space between the array and the bottom surface is adjusted by the space adjusting means. The adjusted array is brought into contact with a biological sample introduced into the chamber. The sample solution is mixed in the chamber. Examples of means for mixing a sample solution include ultrasound, reflux pump, a roller, a space adjusting means, and solenoid.

[0005]

As described in U.S. Patent No. 6,186,659, a sample solution may be mixed by the mechanism whereby superposing substrates on top of each other, introducing the solution into the space between the substrates, generating bubbles in the solution, and causing the bubbles to move.

[0006]

[Problems to Be Solved by the Invention]

According to a conventional method of trickling the sample-containing hybridization solution on the array substrate onto which the probes have been fixed, the duration of hybridization reaction is prolonged as long as approximately 12 hours, and the reaction efficiency is low.

[0007]

The aforementioned U.S. Pat. Publication No. 2001/0046702 and U.S. Patent No. 6,186,659 describe techniques for mixing a solution, wherein a solution is mixed. According to these techniques, however, the flow of the solution on the array substrate becomes inhomogeneous via mixing, and the reaction on the substrate becomes inhomogeneous. This results in unsatisfactory reaction efficiency and varying reaction efficiency depending on the positions of the probes.

[0008]

An object of the present invention is to provide a method for mixing a solution that enables homogeneous reactions on the array substrate and improves the reaction efficiency, and an apparatus used therefor.

[0009]

[Means for Attaining an Object]

The aforementioned object can be attained by causing a planar member to move relative to a base plate member on which a probe-fixed array substrate is provided intervened by a sample solution. Specifically, the solution mixing apparatus of the present invention comprises: a base plate member on which an array substrate is to be provided; a planar member thereon intervened by a space that holds a sample solution; a means for feeding a sample solution to the array substrate, and an actuating means for causing the planar member to move relative to the base plate member. In the method for mixing a solution according to the present invention, a sample solution is introduced into a space between the array and the planar member, and the planar member is caused to move relative to the base plate member on which the substrate is provided, so as to maintain the flow rate of the sample solution at substantially the same level at any spot on the substrate.

[0010]

Thus, the sample solution is caused to move with the use of the planar member, and the sample solution is then homogeneously and effectively fed onto the surface of the array substrate. Accordingly, the reaction can be homogeneously proceeded on the array substrate, and the resulting reaction efficiency can be sufficiently high.

[0011]

Specifically, the planar member may be caused to move relative to the base plate member by fixing either one of the base plate member or planar member and allowing the other member to move or allowing both members to move. Further, such movement may be spinning or linear reciprocation. When the planar member in the region on the array probe-fixed substrate has a circular or polygonal form, the circular plate diameter of the member should be twice or longer than that of the circular region, and the diameter should be twice or longer than the diagonal line of the polygonal region. When the planar member has a square form and linearly reciprocates, the distance of the reciprocation should be twice or longer than the diameter of the probe-fixed circular region or twice or longer than the diagonal line of the polygonal region. Thus, the flow rate of the sample solution becomes substantially the same at any spot on the array substrate, when allowing the planar member to move relative to the base plate member. This results in remarkably improved reaction uniformity.

[0012]

The object of the present invention can also be attained by introducing a sample solution into the installation member holding therein the array substrate and spinning the installation member to generate the centrifugal force. Specifically, a solution mixing apparatus comprises an installation member holding an array substrate on the inner wall thereof, a means for injecting the sample solution into the installation member, and an actuating means for spinning the installation member.

[0013]

By the centrifugal force generated via the spinning of the installation member, the surface of the array substrate, which is opposite from the probe-fixed surface, is pressed against the inner wall of the installation member, and the sample solution is circularly supplied along the inner wall. Thus, the sample solution is homogeneously and effectively fed onto the surface of the array substrate. This results in the proceeding of the homogeneous reactions on the array substrate and satisfactory reaction efficiency.

[0014]

Here, the relationship between a probe and a target substance in a sample solution may be any of nucleic acid-single stranded nucleic acid, nucleic acid-double stranded nucleic acid, antigen-antibody, antibody-antigen, ligand-receptor, receptor-ligand, substrate-enzyme, enzyme-substrate, peptide nucleic acid-nucleic acid, or nucleic acid-peptide nucleic acid. Since the mixing efficiency affects the hybridization results in low-molecular-weight DNA or RNA, any of the aforementioned constitutions can yield satisfactory reaction efficiency and thus is particularly effective.

[0015]

[Embodiments of the Invention]

Examples of the present invention are described below.

[0016]

[Example 1] This example describes a case where a solution is mixed with the use of the constitution shown in Fig. 7. This example involves the use of: (1) a base plate member on which an array substrate 21 is provided; and (2) a planar member provided separated from the array substrate by a space that holds a sample solution, and the planar member 22 spins relative to the base plate member 23. As shown in Fig. 7, the probe-fixed area 21' is located at the center of the substrate. As shown in Fig. 8, a probe 4 of a different type is fixed on the probe-fixed area 21', and a target substance 5 in the solution binds to the probe 4.

[0017]

The sample solution 6 is injected into the space between the array substrate 1 and the planar member 2 with a sample injector 11 (Fig. 3) or through a fluid path 7 communicated with the planar member 2 (Fig. 4). Use of the fluid path 7 can shorten an introduction path for the sample solution, and this can reduce the amount of the sample solution to be injected. Thus, use of the fluid path 7 is more preferable.

[0018]

After the reactions such as hybridization, the sample solution remaining in the space between the array substrate 1 and the planar member 2 is suctioned with a suctions apparatus 15 (Fig. 3).

[0019]

The surface of the planar member 2 that is brought into contact with the sample solution is hydrophilic. Specifically, the planar member is made of glass or metal having on its surface an oxidation film. The surface that is brought into contact with the sample solution is selectively processed so as to attain hydrophilic properties. For example, the surface is processed with an aqueous solution of aminoethylaminopropyltrimethoxysilane (containing an acetic acid catalyst) and is left at room temperature for about 30 minutes. After washed with water, the surface is heated in air at about 110°C for about one hour to introduce an amino group onto the surface. Thereafter, the surface is processed with acetic anhydride in an ethanol solvent at about 50°C for about 30 minutes to displace an amino group on its surface with a carboxyl group. By means of modifying the surface with carboxyl, the surface is negatively charged in the water, i.e. the surface has hydrophilic property. In addition, it can avoid the adsorption of any negatively charged substances, such as DNAs onto the surface of the plate. Because of the hydrophilic surface, the sample solution is guided into the space between the substrate 1 and the planar member 2 via capillary phenomenon.

[0020]

The surface of the planar member and the probe-fixed area are substantially smooth. In order to optimize the reaction, a minute fluid path may be provided, or a groove for capturing bubbles resulting from maintenance at high temperature may be provided.

[0021]

Bubbles would not enter into the space that holds the sample solution between the array substrate and the planar member due to the movement of the planar member. That is, the reaction efficiency of hybridization or the like would not be deteriorated due to contamination with bubbles.

[0022]

A means for spinning the planar member is as shown in Fig. 9. A pair of spinning shafts 24a and 24b is fixed on a pair of small auxiliary plates 27a and 27b, respectively. The small auxiliary plates 27a and 27b each independently comprise a spinning axis connected to the plate 28. The spinning shaft 24a is fixed on the small auxiliary plate 27a, the spinning shaft 24b is fixed on the small auxiliary plate 27b, and each set of the shaft and the plate rotates conjointly. While the large auxiliary plate 28 is rotatably connected to the small auxiliary plates 27a and 27b, it is fixed on the planar member 22, and the large auxiliary plate rotates conjointly with the planar member 22. Further, the large auxiliary plate 28 rotates conjointly with the planar member 22 relatively to the substrate. Upon spinning of the spinning shafts 24a and 24b, the corresponding small auxiliary plates 27a and 27b rotate relatively to the substrate, and the planar member 22 is allowed to spin around the center of the base plate member 22 (and the center of the probe-fixed area 21') instead of around the center of the planar member 22.

[0023]

Fig. 10 is a perspective view showing the positional relationship between the probe-fixed area and the planar member, when the spinning shafts synchronize and rotate by 90 degrees each. The planar member 22 fixed on the large auxiliary plate 28 is located a given space away from the array substrate 21. The small auxiliary plates 27a and 27b are rotatably engaged with the axes 29a and 29b mounted on the large auxiliary plate 28. The axes 29a and 29b are fixed on the small auxiliary plates 27a and 27b, respectively. The spinning centers of the small auxiliary plates 27a and 27b are the centers of the spinning shafts 24a and 24b, respectively, and such spinning centers are separated from the axes 29a and 29b by the distances indicated by the reference numeral 33 in Fig. 10. Accordingly, the planar member 22 mounted on the large auxiliary plate 28 decenters by substantially the same distance indicated by the reference numeral 33 in Fig. 10 and rotates. The amount of such decentering is approximately 10 mm, when the radius of the planar member is 40 mm, the radius of the probe-fixed area is 20 mm, and the radius of the circular small auxiliary plate is 13 mm. Based on the perspective observation, the planar member 22 entirely covers the probe-fixed area 21' and rotates around the probe-fixed area 21' while being in contact with the circumferential frame of the probe-fixed area 21'.

[0024]

Fig. 11 shows the spinning direction 26 of the planar member 22 relative to the probe-fixed area 21' and the relative moving vectors of the planar member 22 at spots 25a, 25b, 25c, and 25d on the probe-fixed area 21'. Fig. 11A shows that the relative moving vectors of the planar member toward the 12 o'clock position on a clock increase from 0 at position 25a to positions 25b, 25c, 25d, and to a maximum value at position 25e. Fig. 11B shows that the relative moving vectors are directed toward the 3 o'clock position on a clock at all positions 25a to 25e. Fig. 11C shows that the relative moving vectors toward the 6 o'clock position on a clock increase from 0 at position 25e to positions 25d, 25c, 25b, and to a maximum value at position 25a. Fig. 11D shows that the moving vectors are directed toward the 9 o'clock position at all the positions 25a-25e. Accordingly, the sum of the relative moving vectors of the planar member 22 becomes 0 at each of positions 25a-25e after each rotation cycle. This indicates that the same may apply to the flow of the solution resulting from the movement of the planar member, and the sum of the relative moving vectors of the solution becomes 0 at each of positions 25a to 25e, and all the spots on the probe-fixed area after each rotation cycle. Specifically, the flow rates of the solutions become substantially the same at all the spots on the probe-fixed area after one rotation cycle.

[0025]

The spinning speed of the planar member is between 0.1 sec and 1 min/cycle, and preferably between 0.9 and 30 sec/cycle.

[0026]

The planar member and the probe-fixed area of the example may take a variety of configurations, with the only limitations being (1) that the area that the base plate member moves is large enough to cover the area that the planar member moves, and (2) that the area that the planar member moves is large enough to cover the probe-fixed area. In the examples of the present invention, the planar member and the probe-fixed area have a circular, square (Fig. 12), rectangular, hexagon (Fig. 11), or any symmetrical shape. In the case of Example 1 shown in Fig. 7, for example, the probe-fixed area and the planar member ($R > 2r$) are circular, and the substrate is rectangular. When both the probe-fixed area and the planar member are circular, for example, the diameter R of the planar member is preferably 4 cm or larger when the radius r of the probe-fixed area is 2 cm.

[0027]

The reaction such as hybridization may be analyzed in the following manner. For example, the sample before the reaction, which contains a target substance binding to a probe, is subjected to processing, such as labeling of the target substance in the sample with a fluorescent substance, the fluorescent intensity is measured after the reaction, and the presence or absence and the amount of the target substance are analyzed. Such analysis may be carried out by incorporating a relevant mechanism to the apparatus of the present invention. Alternatively, another detector may be used following the performance of the reaction using the apparatus of the present invention, unlike the present example.

[0028]

Fig. 5 shows the results of a comparison of the hybridization speeds resulting from a case where the sample solution is spin mixed in the present example and a case where the sample solution is allowed to stand without spin mixing. The ordinate denotes the relative fluorescence intensity, and the abscissa denotes the reaction time (minute). Sufficient fluorescence intensity was obtained in a reaction time of about 10 minutes when spin mixing was carried out. When the solution was allowed to stand, however, the fluorescence intensity after 180 minutes was as low as about a half of that attained when spin mixing was carried out. This indicates that the spin mixing enables the performance of hybridization within a very short period of time. Experimental conditions, such as the buffer composition and the sample DNA probe, were determined in accordance with the hybridization technique described in Nucleic Acids Research, 2002, Volume 30, No. 16, e87. Specifically, the sample DNA used here is 0.1 nM synthetic oligonucleotide (5'-TGACGGAGGTTGTGAGGC-3'; SEQ ID NO: 1) that has eighteen bases and the 5-'end labeled with sulforhodamine 101 (also known as Texas Red). A probe having a sequence complementary to the sequence of the sample DNA and at its 5' end an SH group (5'-GCCTCACAACCTCCGTCA-3'; SEQ ID NO: 2) is fixed on the probe-fixed area. The probe is fixed by first processing the glass substrate with 3-aminopropyltrimethoxysilane and then introducing an amino group onto the surface thereof, in accordance with the aforementioned document. The amino group introduced onto the glass surface was bridged with the SH group of the probe with N-(11-maleimidoundecanoxyloxy) succinimide.

[0029]

Fig. 6 shows the results of inspecting the relationship between the space distance between the substrate and the planar member and the reaction efficiency in the present example. The ordinate denotes the relative fluorescence intensity, and the abscissa denotes the distance (μm) between the substrate and the planar member. The reaction efficiency increases exponentially along with the decrease of the distance, i.e., the decrease of the distance between the substrate and the planar member. A higher reaction efficiency may be attained when the distance is between 50 μm and 100 μm. When the target is double-stranded DNA comprising 10 nucleotides, for example, the length thereof is 24 nm, and the length of each probe nucleic acid is 10 to 100 nm. Thus, the length of the probe nucleic acid-target nucleic acid combination is of 100 nm order, which is ignorable, compared with the distance between the substrate and the planar member of 50 μm to 100 μm. Thus, the distance between the probe and the planar member on the substrate is approximately the same as the distance between the substrate and the planar member.

[0030]

Further, variations in the reaction efficiency resulting from differences in sites on the probe-fixed area were inspected. Specifically, DNA probes having the identical sequence were fixed on various sites on the probe-fixed area, hybridization was carried out while spin mixing the sample solution and while allowing the sample solution to stand still, and the relative standard deviations of the fluorescent intensities were determined (standard deviation/mean). As a result, variations in the fluorescent intensity, i.e., the relative standard deviations, were found to drop to about 1/13, when the sample solution was subjected to spin mixing. This indicates that spin mixing improves the hybridization homogeneity.

[0031]

The apparatus of the present invention comprises a temperature control apparatus 20 (Figs. 2 and 3) for maintaining temperature at a level suitable for reactions such as hybridization. As shown in Figs. 2 and 3, the temperature control apparatus may be provided on top of the planar member 2, or it may be incorporated into the base plate member and provided under the substrate 1. Alternatively, the temperature control apparatus may be provided so as to surround the space containing the planar member and the base plate member. Such techniques may be employed in combination. With the use of the temperature control apparatus, the optimal temperature for the reactions such as hybridization can be maintained, and the solution can be simultaneously mixed. Thus, reaction efficiency can be further improved.

[0032]

[Example 2] This example describes a case where a solution is mixed with the use of the constitution shown in Fig. 1. This example involves the use of: (1) a base plate member 3 on which an array substrate 1 is provided; and (2) a planar member 2 provided on the array substrate intervened by a space that holds a sample solution, and the planar member linearly reciprocates relative to the base plate member. In Example 1 (Fig. 7), the planar member 22 rotates relative to the base plate member 23. In Example 2, however, the planar member 2 moves back and forth toward a given direction. The probe-fixed area 1' is located at the center of the substrate 1.

[0033]

In Example 2, the planar member 2 reciprocates relative to the array substrate 1 and to the base plate member 3 in order to mix the solution on the array substrate 1, particularly in the probe-fixed area 1'. The array substrate 1, the probe-fixed area 1', the planar member 2, and the base plate member 3 can be positioned as shown in Fig. 1A to Fig. 1C.

[0034]

The reciprocation rate of the planar member is between 0.1 sec and 1 min/cycle, as with the case of Example 1. The range between 0.9 sec and 30 sec/cycle is more effective.

The base plate member 3, the planar member 2, and the probe-fixed area 1' of Example 2 are required to satisfy the following conditions: (1) the base plate member is large enough to cover the area that the planar member moves; and (2) the area that the planar member moves is large enough relative to the probe-fixed area. In the examples of the present invention, the planar member or the probe-fixed area has a circular, square, or rectangular form. In the case of Example 2 shown in Fig. 1, for example, the distance that the probe-fixed area 1' is caused to move and the distance that the planar member 2 is caused to move are designated as l and L, respectively, and L is more than twice l (L > 2l). The probe-fixed area has a rectangular form, and the planar member 2 has a square form. When l is 24 mm, for example, L is preferably 48 mm or larger.

[0035]

Techniques for injecting the sample solution into the space between the array substrate and the planar member 2, techniques for suctioning the sample solution after reaction, materials, surface modification, or configuration of the planar member, techniques for analyzing the reaction results, temperature control means, and other conditions are the same as those employed in Example 1, and description thereof is thus omitted here.

[0036]

As with the case of Example 1, sufficient fluorescence intensity was obtained in a reaction time of about 10 minutes when the sample solution was reciprocally mixed. When the solution was allowed to stand still, however, the fluorescence intensity after 180 minutes was as low as about a half of that attained when spin mixing was carried out. This indicates that reciprocal mixing enables the performance of hybridization reaction within a very short period of time.

[0037]

The correlation between the distance between the substrate and the planar member and the reaction efficiency observed in this example was found to be the same as Example 1. That is, the reaction efficiency increased exponentially along with the decrease of the distance, i.e., the decrease of the distance between the substrate and the planar member. A higher reaction efficiency was found to be attained when the distance was between 50 μm and 100 μm.

[0038]

Further, variations in the reaction efficiency resulting from differences in sites on the probe-fixed area were also inspected concerning the case where the sample solution was subjected to reciprocal mixing and the case where the solution was allowed to stand still, as with the case of Example 1. As a result, variations in the fluorescent intensity, i.e., the relative standard deviations, were found to drop to about 1/11, as with the case of Example 1, when the sample solution was reciprocally mixed. This indicates that reciprocal mixing improves the hybridization homogeneity.

[0039]

Accordingly, it was confirmed that Example 2 produced the effects similar to those attained in Example 1.

[0040]

[Example 3] This example describes a case where a solution is mixed with the use of the constitution shown in Fig. 14. This example involves the use of: (1) a flexible array substrate 40; and (2) an installation member 41 that allows the flexible array substrate to be in contact with an inner wall 41b, which is the opposite surface of the surface having a probe-fixed area, that rotates around the center axis 41a, and that holds the sample solution 6 spread on the inner wall 41b, and the sample solution is mixed with the centrifugal force generated by the rotation of the installation member. In Examples 1 and 2, the planar member is caused to move relative to the base plate member and the array substrate provided on the base plate member. In Example 3, however, the installation member rotates.

[0041]

The installation member 41 holds the flexible array substrate 40 therein, and the sample solution 6 is injected into the installation member 41. After the injection of the sample solution 6, a cover 41d is provided on top of the installation member 41 (Fig. 14).

[0042]

Fig. 15 shows a partial cross-sectional view of the installation 41 at the time of the spinning thereof and the flexible array substrate. When the installation member 41 rotates, the flexible array substrate 40 is in contact with the inner wall 41b, which is opposite from the surface comprising the probe-fixed area 42 thereon, and the flexible array substrate 40 is pressed against the inner wall 41b. Fig. 15 shows the flexible array substrate 40 observed from the inside of the installation member 41, as the flexible array substrate 45. The sample solution 43 provided at the bottom 41c of the installation member 41 spreads on the inner wall 41b and covers the surface of the flexible array substrate 40.

[0043]

The high-speed spinning of the installation member increases the relative moving vector of the sample solution to the flexible array substrate 40. This can consequently enhance the reaction efficiency. When the speed is excessively high, however, the probe-fixed area is disadvantageously damaged. Thus, the rotation speed is preferably between 100 rpm and 1,000 rpm, and more preferably between 200 rpm and 400 rpm.

[0044]

In conventional spinning apparatuses, rotation takes place along with the horizontal axis, and the membrane is not always soaked in the solution. According to the present example, however, rotation of the installation member spreads the sample solution on the inner wall 41b, and the sample solution is always present on the inner wall during the rotation. Thus, the surface of the flexible array substrate that is pressed against the inner wall is always covered with the sample solution.

[0045]

In the present example, the flexible array substrate and the probe-fixed area can take various configurations, provided that the flexible array substrate is small enough to be provided on the inner wall of the installation member. The probe-fixed area has a circular, rectangular, hexagonal, or symmetrical form. For example, the probe-fixed area has a square form, and the flexible array substrate has a rectangular form. The installation member may have a cylindrical or conical form.

[0046]

Techniques for injecting the sample solution into the space between the array substrate and the planar member 2, techniques for suctioning the sample solution after reaction, materials, surface modification, or configuration of the planar member, techniques for analyzing the reaction results, temperature control means, and other conditions are the same as those employed in Example 1, and description thereof is thus omitted here.

[0047]

In this example, sufficient fluorescence intensity was obtained in a reaction time of about 30 minutes when the sample solution was reciprocally mixed. When the solution was allowed to stand, however, the fluorescence intensity after 180 minutes was as low as about a half of that attained when spin mixing was carried out. This indicates that centrifugal mixing enables the performance of hybridization reaction within a very short period of time.

[0048]

Further, variations in the reaction efficiency resulting from differences in sites on the probe-fixed area were also inspected as with the case of Example 1. As a result, variations in the fluorescent intensity, i.e., the relative standard deviations, were found to drop to about 1/6 as with the case of Example 1, when the sample solution was mixed. This indicates that centrifugal mixing improves the hybridization homogeneity.

[0049]

Thus, it was confirmed that Example 3 also produced the effects similar to those attained in Example 1.

[0050]

[Sequence Listing]

SEQUENCE LISTING

<110> HITACHI, LTD.

<120> SOLUTION MIXING APPARATUS AND SOLUTION MIXING METHOD

<130> H201102

<160> 2

<210> 1
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<212> DNA
<213> Artificial Sequence

<223> DNA probe

<400> 1
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<210> 2
<211> 18
<212> DNA
<213> Artificial Sequence

<220>

<223> DNA probe

<400> 2

gcctc acaac ctccg tca

gcctcacaac ctccgtca

[Brief Description of the Drawings]

Fig. 1 schematically shows a principle part of a solution mixing apparatus of Example 2 according to the present invention.

Fig. 2. is a partial cross-sectional view of a principle part of a solution mixing apparatus of Example 2 according to the present invention.

Fig. 3 shows an example of a sample solution injector and that of a sample solution suction apparatus according to the present invention.

Fig. 4 shows an example of a sample solution injector according to the present invention.

Fig. 5 shows the correlations between the reaction speed and the fluorescence intensity, where reciprocal mixing is or is not carried out, of Example 2 according to the present invention.

Fig. 6 shows the correlations between the distance between the substrate and the planar member and the reaction efficiency, where reciprocal mixing is or is not carried out, of Example 2 according to the present invention.

Fig. 7 schematically shows a principle part of a solution mixing apparatus of Example 1 according to the present invention.

Fig. 8 is a partial cross-sectional view of a principle part of a solution mixing apparatus of Example 1 according to the present invention.

Fig. 9 schematically shows a means for spinning the planar member of Example 1 according to the present invention.

Fig. 10 is a perspective view showing the positional relationship between the probe-fixed area and the planar member, when 2 spinning shafts of Example 1 according to the present invention synchronize and rotate by 90 degrees each.

Fig. 11 schematically shows the spinning direction of the planar member of Example 1 according to the present invention and the relative moving vector of the planar member at the probe-fixed area.

Fig. 12 schematically shows an example wherein the probe-fixed area of Example 1 according to the present invention has a square form.

Fig. 13 schematically shows an example wherein the probe-fixed area of Example 1 according to the present invention has a hexagonal form.

Fig. 14 schematically shows a principle part of a solution mixing apparatus of Example 3 according to the present invention.

Fig. 15 is a partial cross-sectional view of a principle part of a solution mixing apparatus and an array substrate of Example 3 according to the present invention.

Fig. 16 schematically shows a prior art of a solution mixing apparatus.

[Description of Reference Numerals]

1: array substrate
1': probe-fixed area
2: planar member
3: base plate member
4: probe
5: target substance
6: sample solution
7: fluid path
11: sample solution injector
15: suction apparatus
21: array substrate
21': probe-fixed area

22: planar member

23: base plate member

24a, 24b: shaft

25a, 25b, 25c, 25d: spots on probe-fixed area 21'

26: spinning direction of planar member

27a, 27b: small auxiliary plate

28: large auxiliary plate

29a, 29b: axis

30a, 30b: moving direction of spinning shaft

31a, 31b: moving direction of small auxiliary plate

32: moving direction of planar member

33. distance between spinning center of small auxiliary plate and axis center

34: bottom

35: cover

36: solution port

37: space control means

38: array provided area

39: chamber

40: flexible array substrate

41: installation member

41a: center axis

41b: inner wall

41c: bottom

41d: cover

42: probe-fixed area

43: sample solution

44. cavity

45: flexible array substrate

[Designation of Document] Abstract

[Abstract]

[Objective] To provide a method for mixing a solution that enables homogeneous reaction on an array substrate and improves reaction efficiency and an apparatus therefor.

[Means for Solving the Problems] In such an apparatus, a planar member provided on a substrate intervened by a sample solution is caused to move relative to a probe-fixed array substrate and to a base plate member on which the array substrate has been provided. A solution is mixed so as to maintain the flow rate at substantially the same level at any spot on the array substrate.

[Effects] The sample solution is caused to move using the planar member to homogeneously and effectively feed the solution onto the array surface. This enables the homogeneous reaction on the array substrate and yields high reaction efficiency.

[Selected Diagram] Fig. 7

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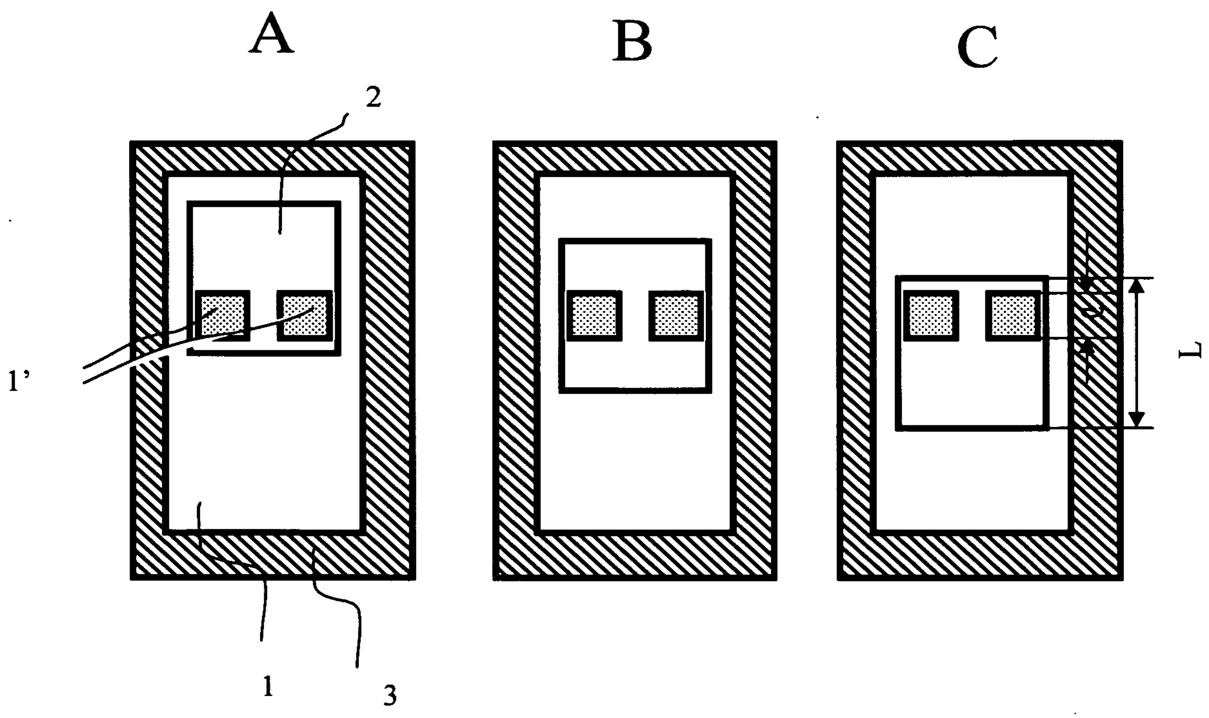


Fig. 1

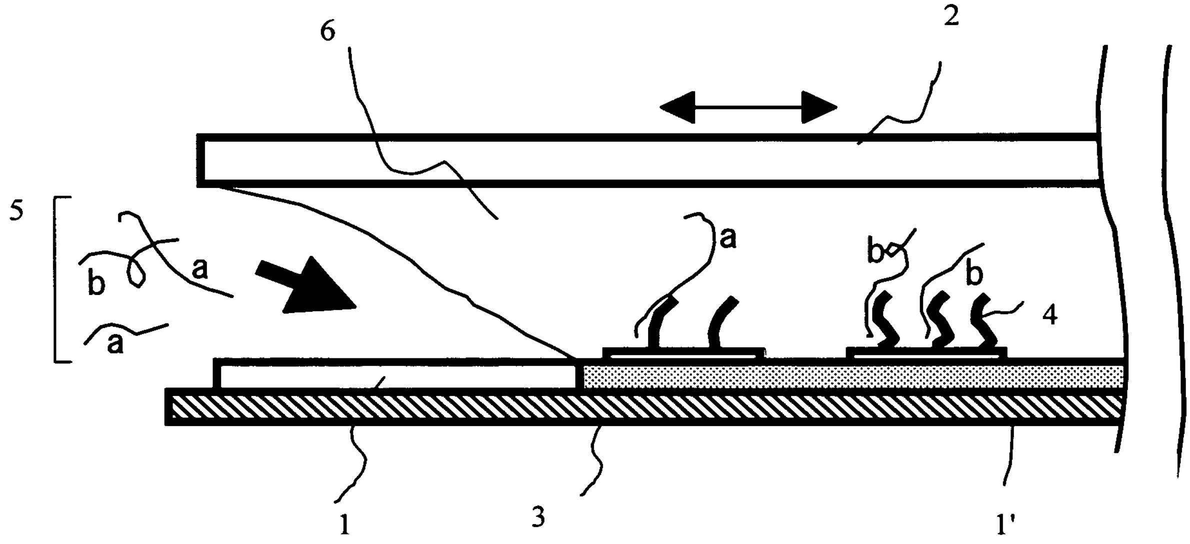


Fig. 2

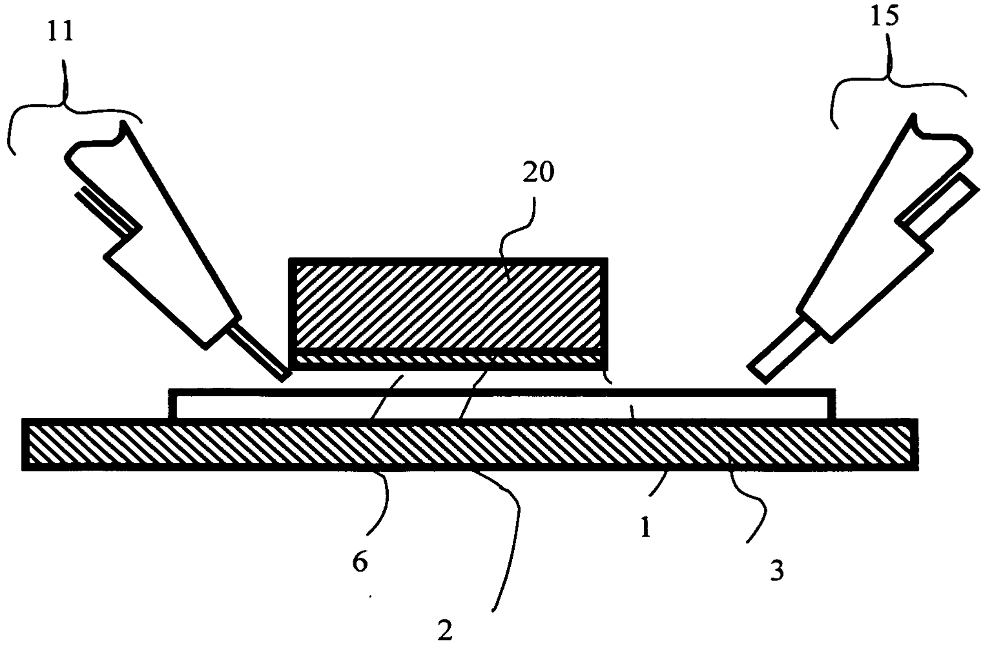


Fig. 3

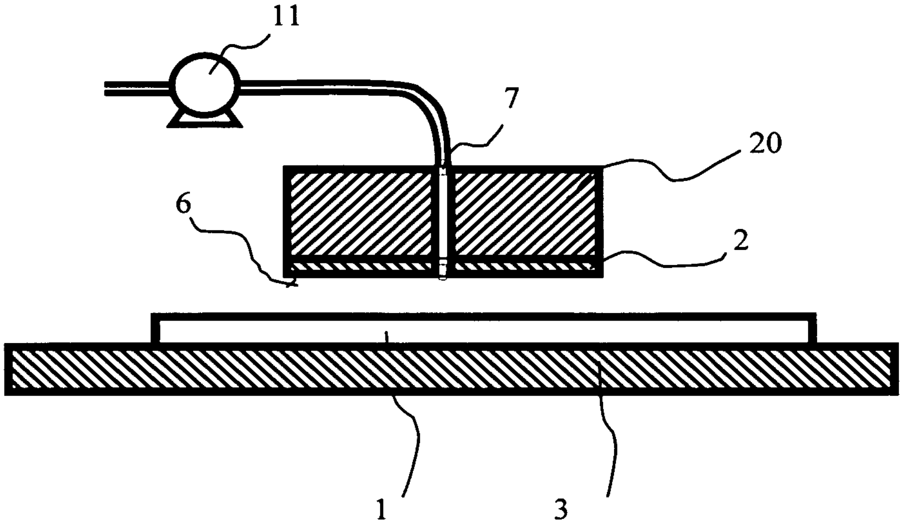


Fig. 4

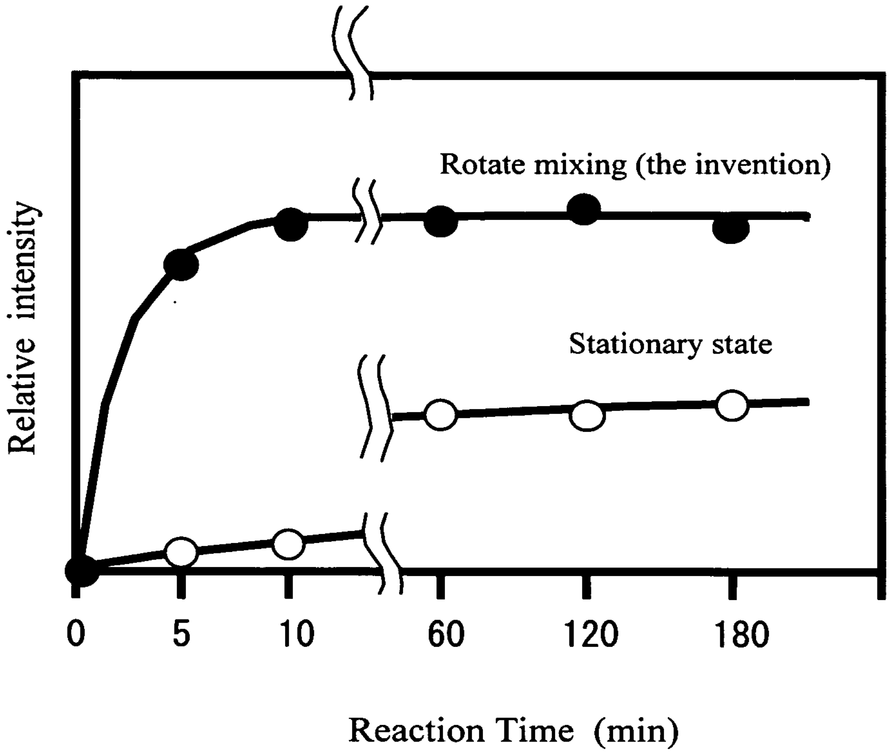

Rotate mixing (the invention)
Stationary state
Relative intensity
0
5
10
60
120
180
Reaction Time (min)

Fig. 5

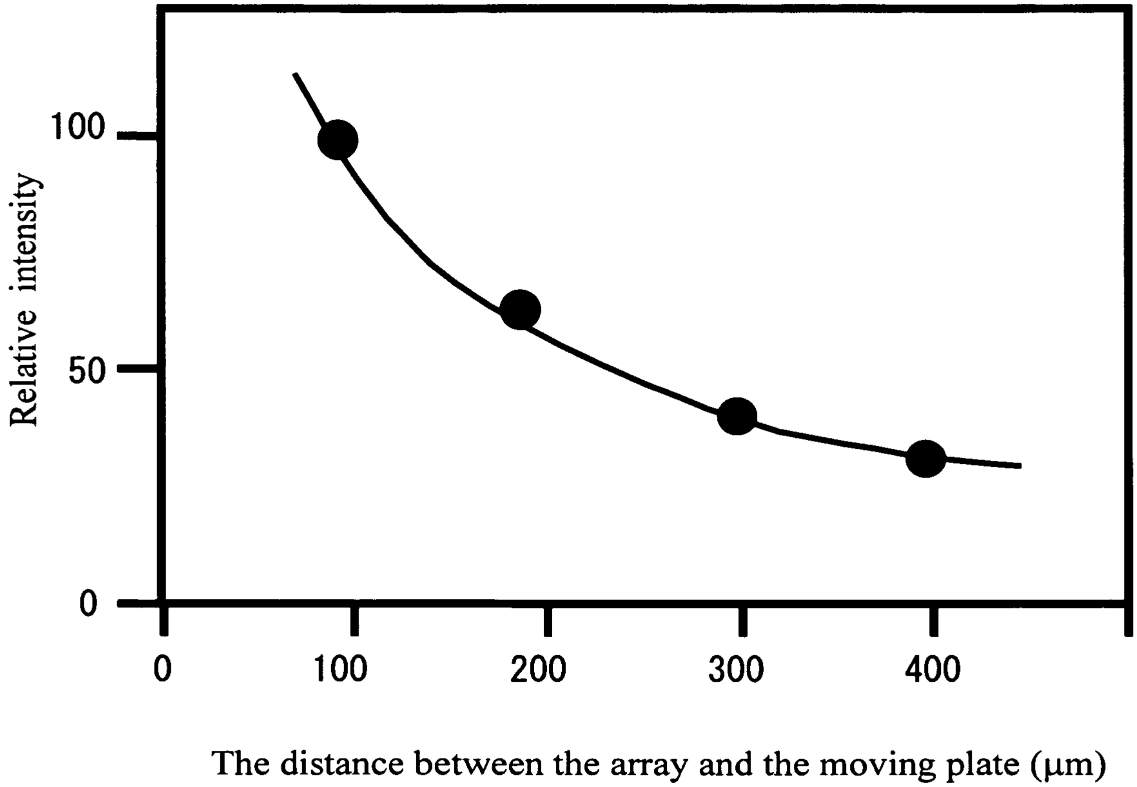

100
50
0
Relative intensity
0
100
200
300
400
The distance between the array and the moving plate (μm)

Fig. 6

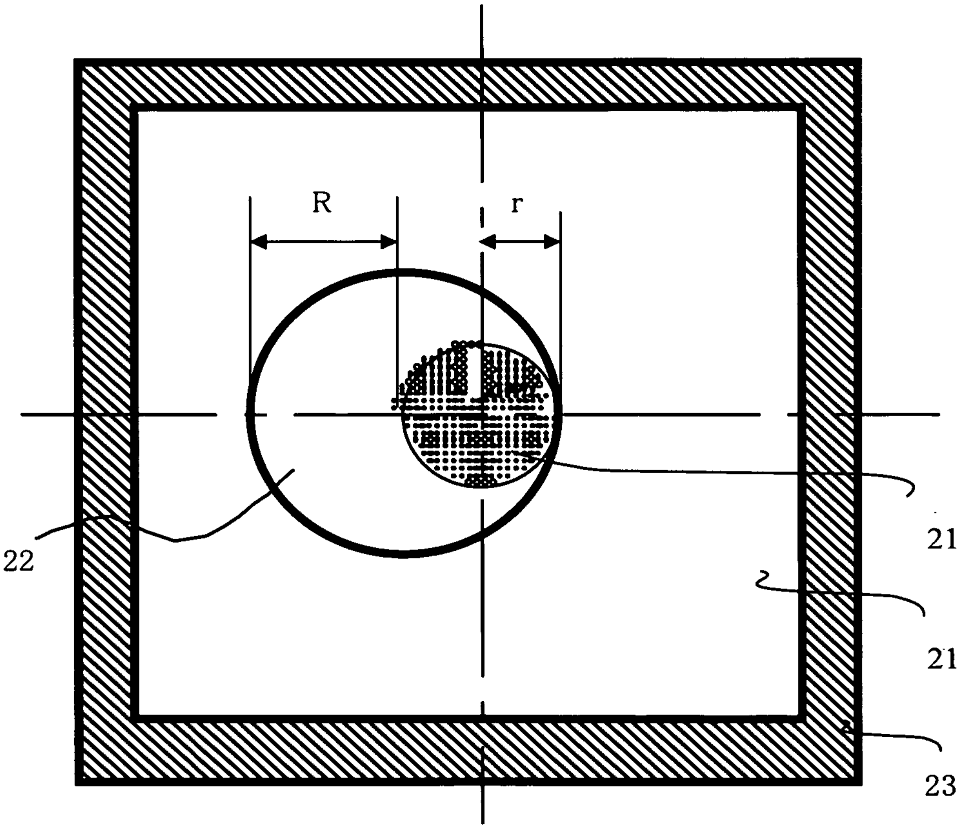


Fig. 7

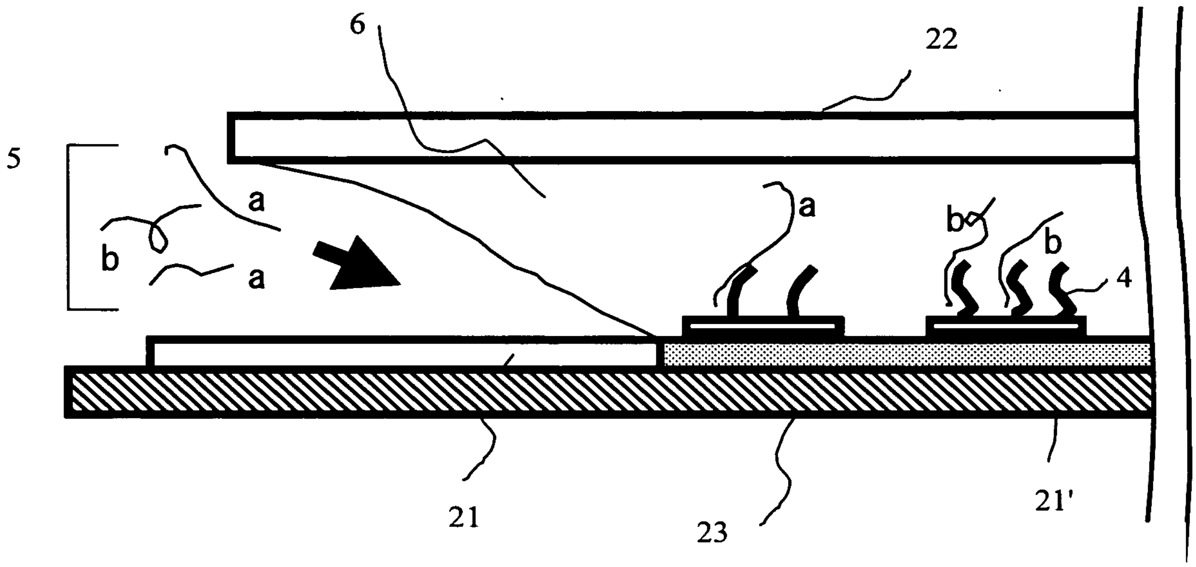


Fig. 8

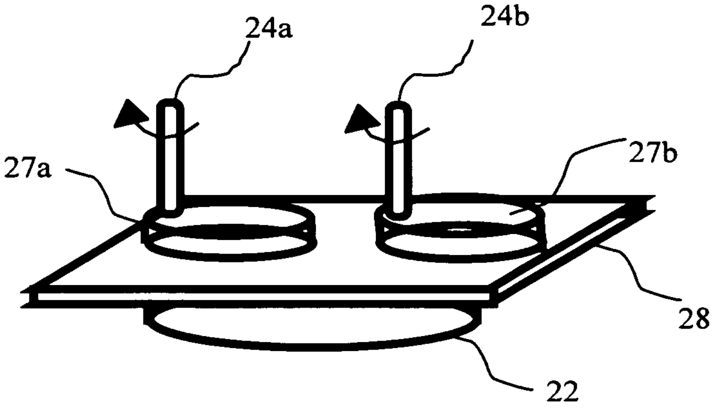


Fig. 9

Fig. 10

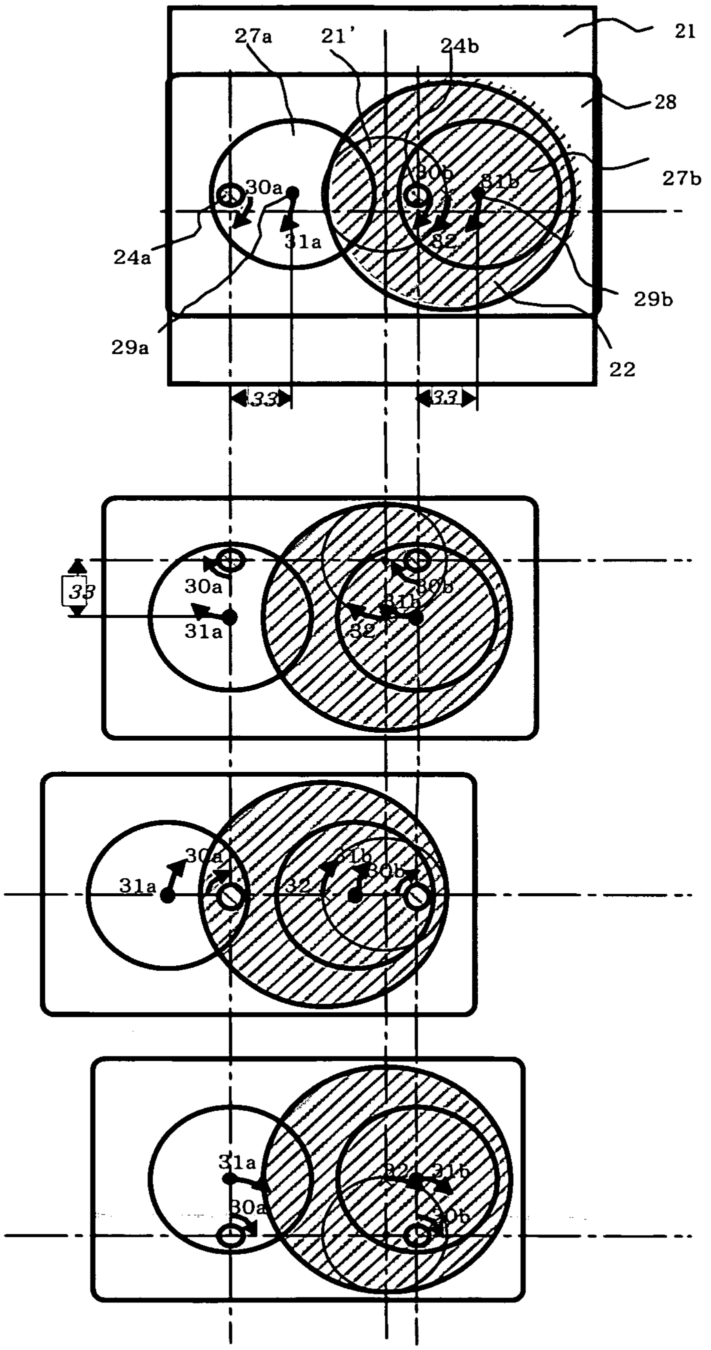
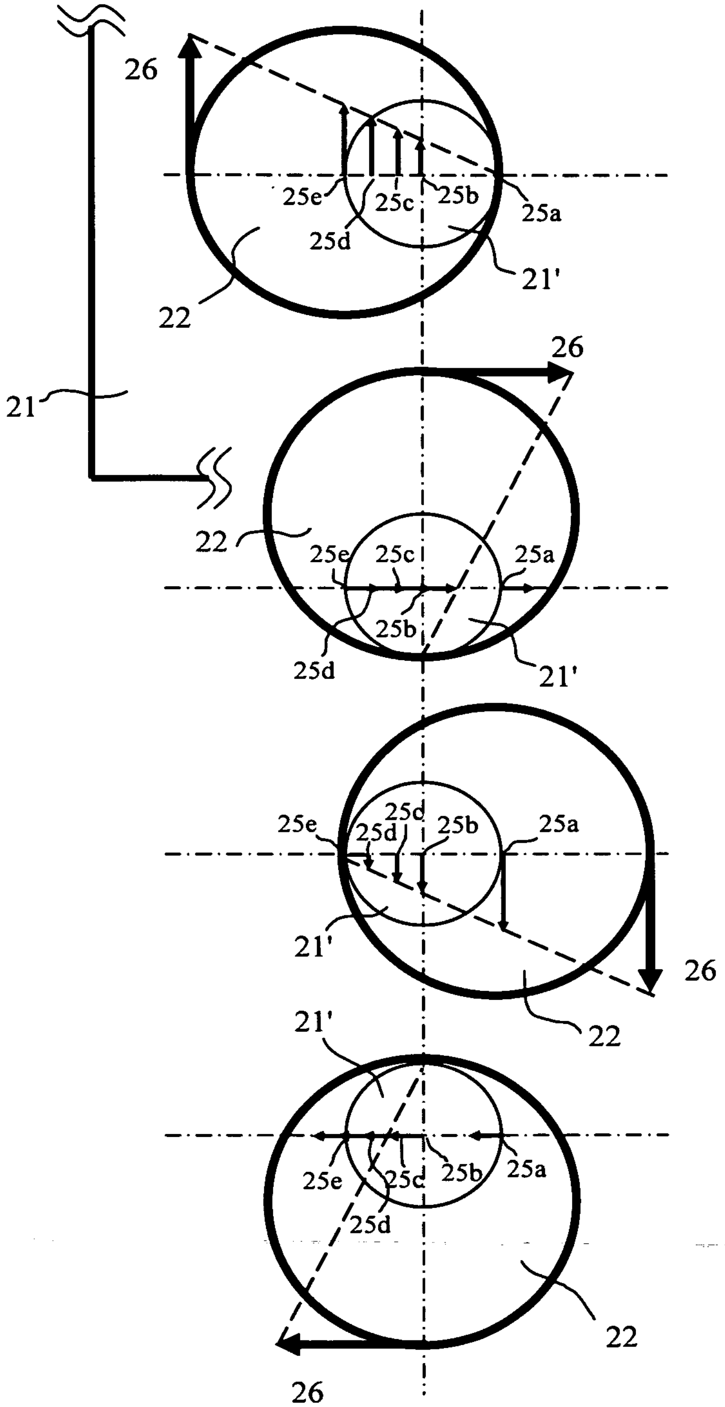


Fig. 11



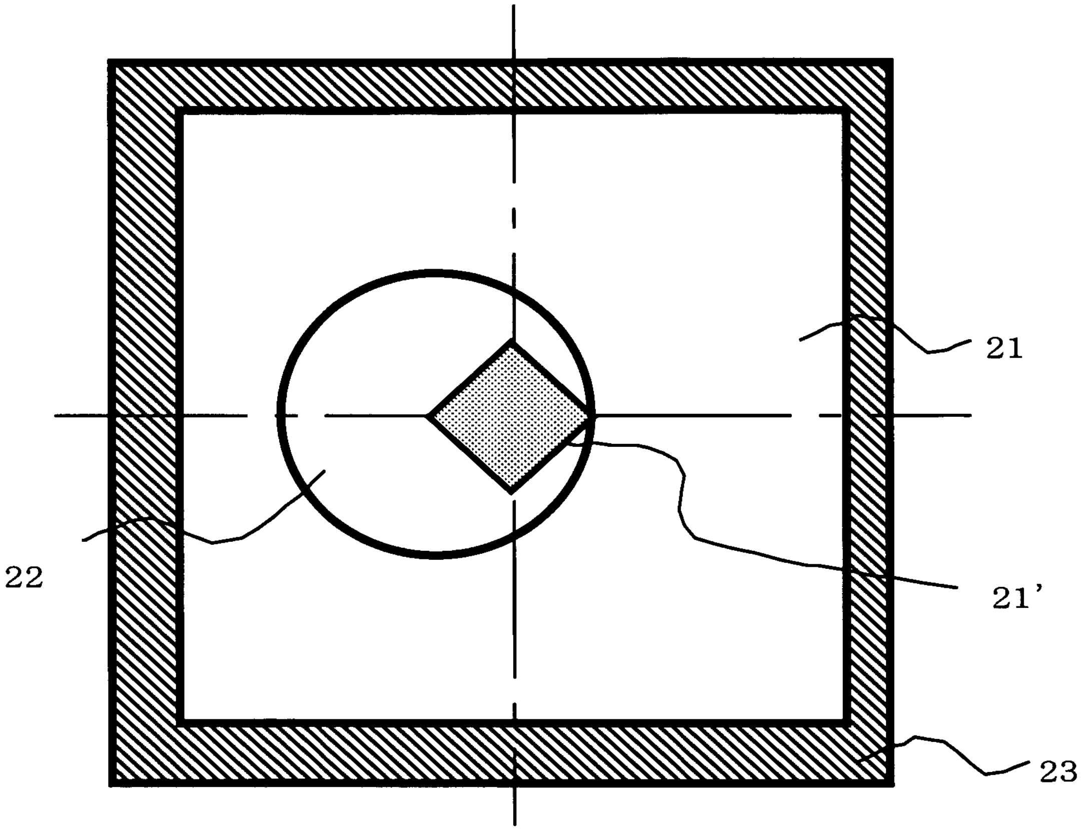


Fig. 12

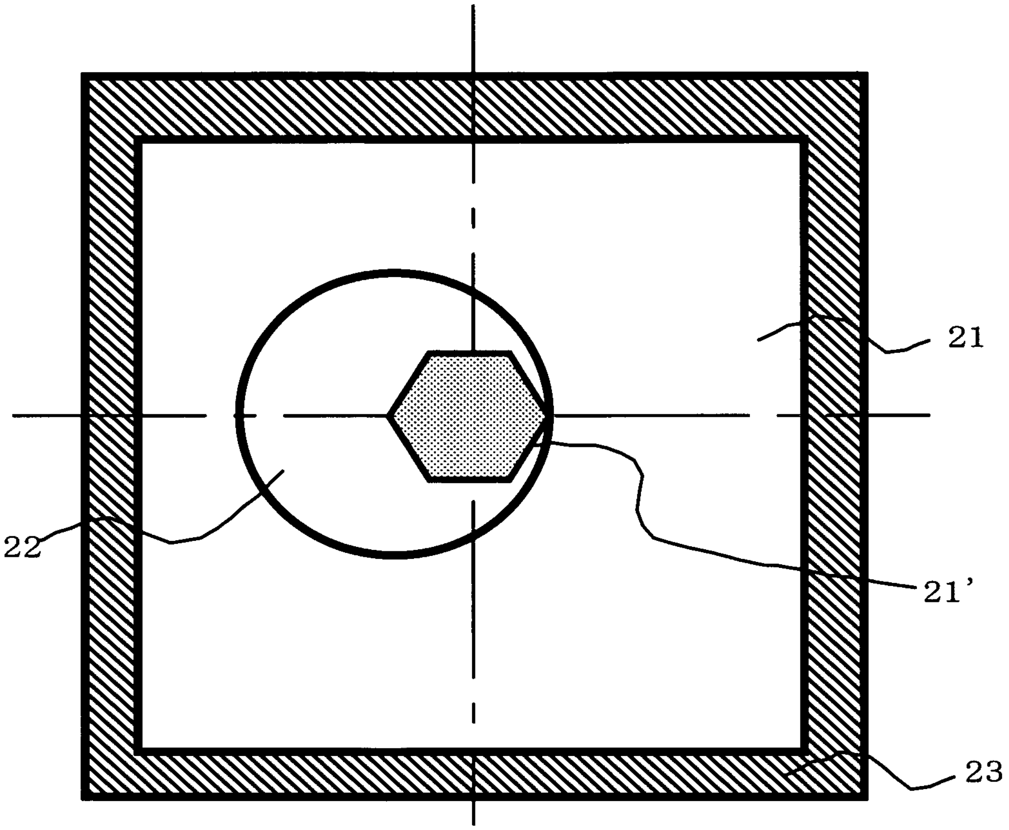


Fig. 13

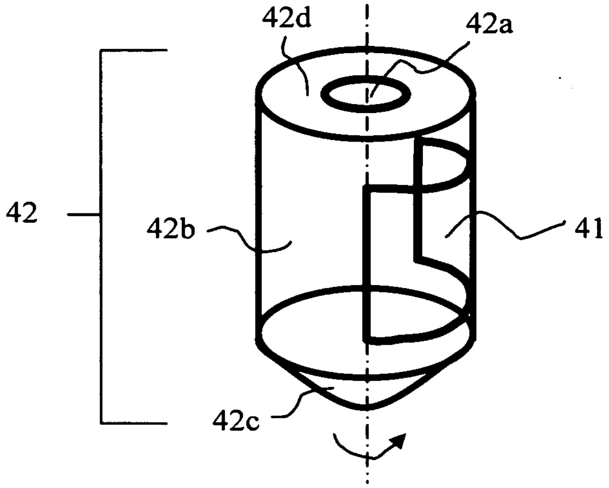


Fig. 14

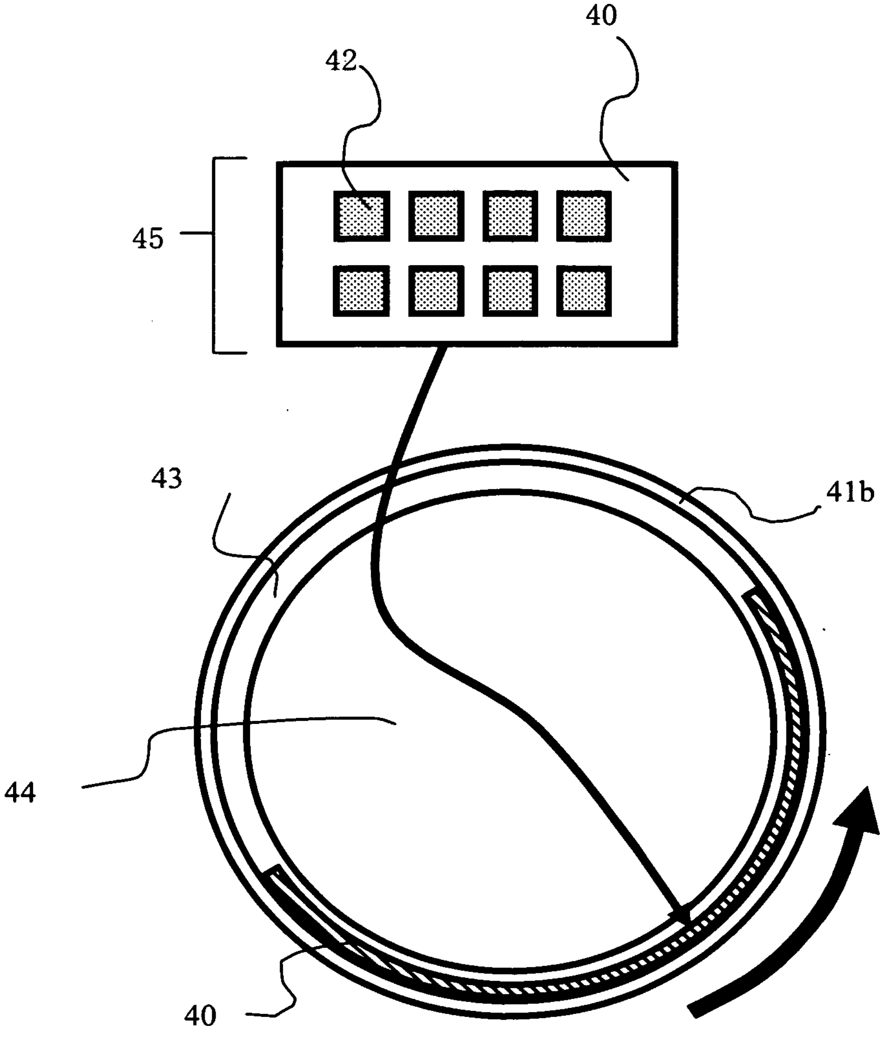


Fig. 15

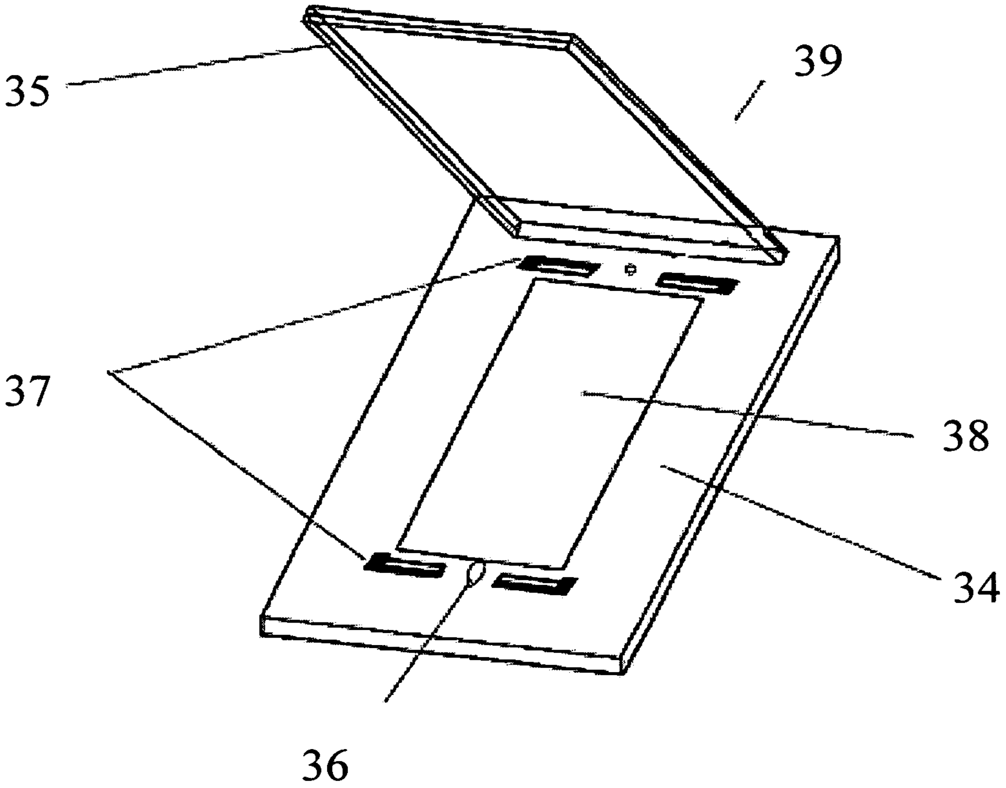


Fig. 16